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Phytate, zinc, iron and calcium content of common Bolivian food, and implications for mineral bioavailability



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ABSTRACT

The content of zinc, iron, calcium and phytate in the 16 most consumed foods from 5 villages in a tropical rural area of Bolivia was analyzed. The food items were selected according to a completed food frequency questionnaire. Minerals were analyzed by atomic absorption and phytates by HPIC chromatography. The molar ratios of phytate:mineral are presented as indication of the mineral bioavailability. Within the analyzed food, quinoa is a potential source of minerals: zinc 3.65, iron 5.40 and calcium 176 mg/100 g; however, it also has the highest content of phytate 2060 mg/100 g. Cereals and legumes showed high concentration of phytates (from 142 to 2070 mg/100 g), roots and tubers have lower concentrations (from 77 to 427 mg/100 g). In general, both phytate contents and molar ratios Phy:Zn (phytate:zinc), Phy:Fe (phytate:iron) and Phy:Ca (phytate:calcium) in most of the analyzed foods were at levels likely to inhibit the absorption of these minerals. Significant positive associations ($p < 0.01$) were found between the level of phytate and minerals in food, for zinc ($r = 0.714$), iron ($r = 0.650$) and calcium ($r = 0.415$). The results compared to data from USA or from Bolivia showed some discrepancies, confirming the need for more reliable data for dietary evaluations and interventions.

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1. Introduction

In the rural areas of developing countries, the diets are mainly plant-based with a limited amount of animal-source foods, including dairy products. These types of diet usually have a low bioavailability of minerals (mainly zinc, iron and calcium) due to the presence of absorption inhibitors such as phytates, tannins, oxalates and others (Lönnnerdal, 2002; Sandberg, 2002). It has been reported that phytate (myo-inositol-6-phosphate) is the main inhibitor of zinc absorption and that it affects the absorption of

other divalent minerals, mostly iron and calcium. Phytate is mainly found in cereal grains, legume seeds and, in a lower concentration, in tubers and roots (Connie and Srimathi, 2001; Lönnnerdal, 2000, 2002).

The inhibitory effect of phytates on the absorption of minerals is due to the formation of insoluble and indigestible phytate–mineral complexes in the gut (Sandstrom, 1997). This negative effect depends not only on the amount of phytate in the food, but also on the molar ratios of phytate:mineral, which have already been studied, and desirable molar ratios of phytate:mineral are suggested as an indicator of the adequate bioavailability of the minerals (Hotz and Brown, 2004; Hotz et al., 2003). The suggested phytate:zinc molar ratio (Phy:Zn) is <15 , although it has been seen that even Phy:Zn ratios between 5 and 15 have a negative effect over the zinc bioavailability (WHO, 1996). For diets high in both phytate and calcium, the molar ratio Phy*Ca:Zn (phytate*calcium:zinc) is more useful for assessing the zinc bioavailability. In

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this case molar ratios higher than 200 are said to impair zinc absorption (Bindra et al., 1986); high amounts of calcium may exacerbate the inhibitory effect on zinc absorption due to complexes of phytate–calcium–zinc being even less soluble (Bindra et al., 1986). The desirable phytate:iron (Phy:Fe) ratio is <1 (Hurrell, 2004), and for phytate:calcium (Phy:Ca) it is <0.17 (Umeta et al., 2005). Ratios above the desirable values indicate that the bioavailability of the mineral is low and highly affected by the phytate content.

The diet in the rural population of Chapare, Bolivia has previously been reported to be a plant-based diet, with small contributions of animal-source foods or dairy products (Lazarte et al., 2012, 2013). In these studies, the dietary intake was evaluated by the food photography 24-h recall method. Mineral intakes were calculated based on the nutrient database for standard reference from the USA (USDA, 2001), since there is a lack of reliable data of prepared food in the Bolivian food composition table (INLASA, 2005). Besides this table reports only the zinc, iron and calcium content of a few foods, and most of the data is not experimental but rather an average of different databases found in the Latin American food composition table. Moreover, there is a lack of information about the phytate content and therefore the mineral bioavailability of food, not only in Bolivia but also in all of Latin America.

Given the paucity of reliable analyzed data, it becomes a very important challenge to obtain more data concerning the content of essential minerals such as zinc, iron and calcium, as well as of the main inhibitor, phytate, in the most consumed foods. This is desirable in order to improve dietary evaluations and have a more informed approach towards the relative mineral bioavailability of the diets. Data of mineral content are also important for further evaluations of trace element-intake and -deficiencies, which are of great consequence, since as it has been reported that in phytate-rich diets the bioavailability of zinc, calcium and iron is markedly depressed in humans and animals (Scholz-Ahrens et al., 2007), leading to mineral deficiencies. Zinc and iron deficiency is still one of the main problems related to nutrition in developing countries. Deficiencies of zinc and iron may impair the immune system, compromising the body's resistance to various infections and diseases. Adequate intake of these essential micronutrients is important for ensuring the optimal growth and development of infants and children and, in general, for a healthy human nutritional status (Konishi et al., 2004). Zinc and iron are the micronutrients that are both most often found to be deficient; this is expected because they have a similar distribution in the food and they have common inhibitors that affect their bioavailability in similar ways (Gibson and Hotz, 2000).

Therefore, the aims of this study were firstly to identify the most consumed food in Chapare, a rural tropical area of Bolivia, and secondly to provide data concerning the content of zinc, iron, calcium and phytate in representative food samples from the area. Results are presented for the prepared food in dry weight, and the moisture content is also presented. Finally, the aim was to estimate the inhibitory effect of phytate on the mineral bioavailability by calculating the corresponding phytate:mineral ratios in the most commonly consumed food.

2. Materials and methods

2.1. Food frequency questionnaire (FFQ)

In order to identify the food items that are most consumed in Chapare, a rural tropical area of Bolivia, a food frequency questionnaire was first carried out among 65 volunteers in the villages of Eterazama and Villa Tunari. The food frequency questionnaire was designed to provide qualitative information

about the most consumed food in the area, following the guidelines found in Gibson (2005). The questionnaire includes a total of 72 food items; trained interviewers asked the respondents to identify the frequency of the food items consumption in a scale of 4 points: each day; often (3 times per week); seldom (once or twice per month); or never.

2.2. Food sampling and preparation of the samples for analysis

Two samples each of the 15 most consumed foods were purchased in the main market of 5 villages in Chapare (a total of 150 samples). The villages selected to represent the whole region were: Ivirgarzama, Chimore, Shinaota, Villa Tunari and Eterazama; additionally 2 samples of one more food item were purchased in two markets in the city of Cochabamba (4 more samples). Approximately 1 kg of each food item was purchased in January 2012; the samples were transported to the Food and Natural Products Center in San Simón University Cochabamba, Bolivia. In laboratory conditions, each sample was cooked individually, following the same preparation methods as the ones commonly used when these foods are consumed, except for wheat flour that was analyzed raw.

The food products were cleaned and peeled if necessary, then boiled on a stove (hot plate electric stove) until the tissue was soft. Two portions of 5 g per sample were separated for moisture analysis, according to the procedure described in AOAC (1995), measuring the indirect removal of water, for which samples were dried at 105 °C (Heating oven; model ED23, Binder, Tuttlingen, Germany) until constant weight. Portions of 200 g per sample were then freeze-dried (freeze dryer; model Christ Alpha 2–4 LD, SciQuip Ltd, Shropshire, UK); approximately 30 g of each dried sample was ground to a fine homogenous powder using an acid-washed mortar and pestle to avoid any mineral contamination for the further analysis of zinc, iron and calcium. Portions of 100 g of

Table 1

English and scientific names of food samples collected, as well as preparation methods.

Food names and description	n	Scientific names	Preparation method
Cereals			
Rice, white medium grain, polished	10	<i>Oryza sativa</i>	Boiled ^a
Maize white	10	<i>Zea mays</i>	Boiled
Wheat grain	10	<i>Triticum aestivum</i>	Boiled
Wheat flour, white	10		
Bread, white 100% white wheat flour	10		
Noodles, based on white wheat flour and eggs	10		Boiled
Quinoa	10	<i>Chenopodium quinoa</i>	Boiled
Tubers			
Cassava	10	<i>Manihot esculenta</i>	Boiled
New cocoyam	10	<i>Xanthosoma sagittifolium</i>	Boiled
Imilla potatoes (imilla)	10	<i>Solanum tuberosum</i>	Boiled
Potatoes (runa)	10	<i>Solanum tuberosum</i>	Boiled
Chuño, traditional freeze-dried potatoes	10	<i>Solanum tuberosum</i>	Boiled
Legumes			
Fava beans	10	<i>Vicia fava</i>	Boiled
Lentils	10	<i>Lens esculenta</i>	Boiled
Peanuts	4	<i>Arachis hypogaea</i> L.	
Others			
Plantains	10	<i>Musa × paradisiaca</i>	Boiled

^a Boiled for about 15 min (depending on the food) until the tissue was soft.

dried sample were transported to Sweden for the analysis of phytate content. All the analyses were made in duplicate. Table 1 presents the common names, scientific names and preparation methods of each food.

2.3. Mineral and phytate analysis

For the mineral analysis, the chemicals used were nitric acid and hydrogen peroxide (TraceSELECT for trace analysis, FLUKA Sigma–Aldrich Co., St. Louis, MO, USA). To avoid interferences, all the glassware was properly washed, immersed in 5% nitric acid solution overnight, doubly rinsed with de-ionized water before use, and non-metallic accessories were used, for example, plastic spatulas. Approximately 500 mg of each ground sample was weighed and digested with the chemicals in Teflon vessels in a microwave reaction system (Model Multiwave PRO, Anton Paar Co., Ashland, VA, USA). After digestion, samples were diluted to 25 mL with de-ionized water. Zinc, iron and calcium were quantified by flame atomic absorption spectrometry with air–acetylene flame (Model AAnalyst 200, Perkin Elmer Corp., Waltham, MA, USA) at 213.9, 248.3 and 422.7 nm wavelengths for each mineral respectively. For the calcium determination, lanthanum oxide (1%w/v) (Sigma–Aldrich Co., St. Louis, MO, USA) was added to standards and samples before analysis in order to suppress phosphorus interference.

A calibration curve of 5 points was prepared for each mineral (100–2000 µg/l) from certified Atomic Absorption Standard solutions (1000 ppm) (Pure standards for atomic absorption, Perkin–Elmer Corp., Waltham, MA, USA). The limit of detection was from 15–30 µg/l for zinc and iron and 60–80 µg/l for calcium. To validate the analysis, certified reference materials for trace elements BCR[®] were used: rice flour (IRMM 804 FLUKA Sigma–Aldrich Co., St. Louis, MO, USA), for zinc the certified value is 23.1 ± 1.9 mg/kg and the analyzed was 22.8 ± 0.5 mg/kg and bovine liver (BCR185R FLUKA Sigma–Aldrich Co., St. Louis, MO, USA), where the certified value for zinc is 138.6 ± 2.1 mg/kg and the analyzed was 136.1 ± 3.2 mg/kg.

For quality control: in each batch of microwave digestion (16 Teflon vessels), one vessel was used for digestion of a certified reference material, and one vessel was used as a blank analysis, containing the nitric acid and peroxide hydrogen but not the samples; these were digested together with the food samples contained in the other 14 vessels. The mineral content of the reference materials and blanks was determined by atomic absorption at the same time as the food samples to check the precision and accuracy of the procedures. Two standards from the standard curve were controlled after each ten measurements. The relative standard deviation (RSD %) was below 5% for each measurement.

Phytate was analyzed as inositol hexaphosphate InsP_6 , in all of the samples, by high-performance ion chromatography (HPIC) according to the method described by Carlsson et al. (2001). Approximately 500 mg of the samples previously dried and ground were weighed and extracted with 0.5 M HCl for 3 h at room temperature (20 °C) under magnetic stirring. The extracts were frozen overnight, thawed and centrifuged at $12,000 \times g$ for 10 min; the supernatants were decanted, and 50 µL of supernatants were injected and analyzed by HPIC with a HPIC Omni Pac PAX-100 (4 mm \times 250 mm) analytical column, and a PAX-100 (4 mm \times 50 mm) guard-column (Columns from Dionex Corp., Sunnyvale, CA, USA). The inositol phosphates were detected and quantified after a post-column reaction with $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ (Sigma–Aldrich Co., St. Louis, MO, USA), the absorbance was monitored at 290 nm using UV detection (Waters 486, tunable absorbance detector, Massachusetts, USA). All the reagents were of analytical grade (Sigma–Aldrich Co., St. Louis, MO, USA), and

de-ionized water was used. The limit of detection of the method was previously detected as 13 mg/100 g (Carlsson et al., 2001).

The results of zinc, iron, calcium and phytate concentrations in each of the analyzed food items are presented on dry weight (DW) basis as the mean \pm SD (mg/100 g DW), and the moisture content as percentage is also presented for each food.

2.4. Estimation of relative mineral bioavailability

In order to estimate the relative bioavailability of zinc, iron and calcium and to give an indication of the inhibitory effect of phytates on the bioavailability of these minerals in the food items, the molar ratios $\text{Phy}:\text{Zn}$, $\text{Phy}:\text{Fe}$, $\text{Phy}:\text{Ca}$ and $\text{Phy}^*\text{Ca}:\text{Zn}$ were calculated. As molecular weight of phytate 660.3 g/mol was used.

3. Results

3.1. Food frequency questionnaire (FFQ)

The food consumption frequencies of animal-source food, cereals, tubers and legumes are presented in Figs. 1–4; the bars indicate the type of food and the percentages of consumption at the levels of: each day, often, seldom and never. The most common animal-source food was egg, followed by chicken and beef (Fig. 1). The consumption of dairy foods was very low, only 9% of the volunteers consumed milk often, none of them each day.

The most consumed cereal products (Fig. 2) were rice and bread; the first seven food items (rice, bread, noodles, maize, wheat flour, wheat grain and quinoa) from this group were selected for the mineral and phytate analysis. The most consumed tubers and roots (Fig. 3) were potatoes (2 varieties, imilla and runa), cassava, new cocoyam and chuño; all of these were selected for analysis. Regarding legumes (Fig. 4): fava beans, lentils and peanuts were the most consumed, and selected for analysis. Additional information about the frequency of consumption of vegetables, fruits and types of fat is presented in Table 2. Of these foods, plantain was selected for the analysis, because it is highly consumed and the portion size consumed is bigger compared to that of the other vegetables or fruits; besides, it is produced in the tropical area.

3.2. Mineral and phytate analysis

The results of, zinc, iron, calcium and phytate content in dry weight and moisture percentages are presented in Table 3. The zinc content was the highest in legumes: fava beans, lentils and peanuts (3.33–4.64 mg/100 g), followed by cereals: quinoa, wheat grain, maize, noodles, wheat flour, rice (1.52–3.65 mg/100 g) and, finally, bread (1.00 mg/100 g). Among the tubers, new cocoyam (2.32 mg/100 g) had the highest amount of zinc followed by cassava (1.48 mg/100 g). The content of zinc in potatoes was low, and the lowest in chuño (0.94–1.13 mg/100 g).

Iron concentration was the highest in lentils and quinoa (6.43 and 5.40 mg/100 g), followed by the content in wheat flour, which was comparable to the content in bread, noodles, fava beans and peanuts (2.54–5.24 mg/100 g). Among the tubers, the highest iron content was found in chuño (2.14 mg/100 g) followed by new cocoyam, and then potatoes, and the lowest was to be found in cassava (0.83–1.84 mg/100 g).

Calcium content was the highest in bread and quinoa (176–187 mg/100 g); the other cereals: noodles, wheat grain, wheat flour, rice and maize had lower values (21–87 mg/100 g). The calcium content in lentils and fava beans (115–131 mg/100 g) was similar to the content in chuño, new cocoyam and cassava (93–120 mg/100 g). Peanuts had a lower content (50 mg/100 g) and the

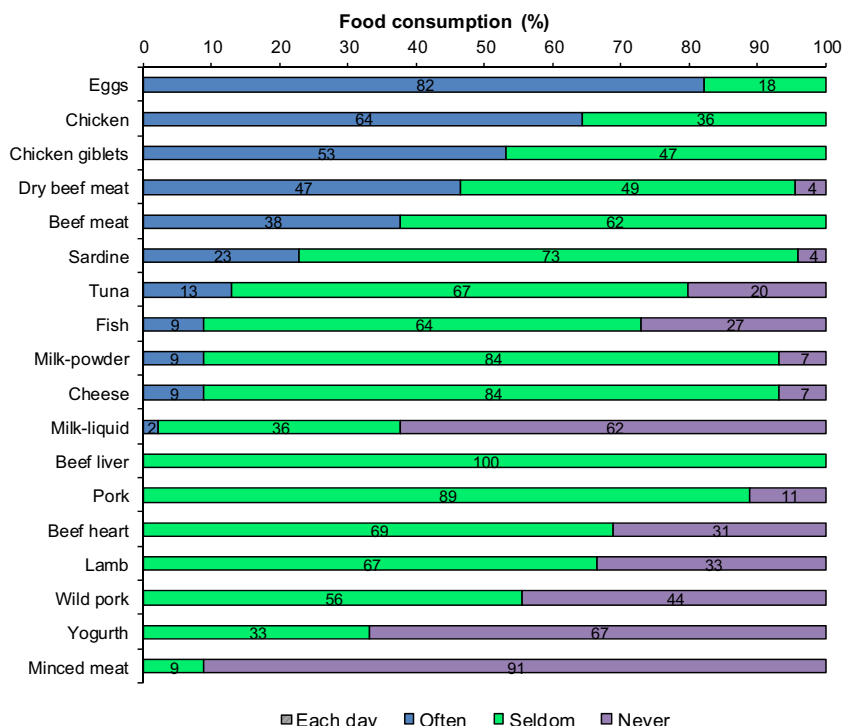


Fig. 1. Frequency of animal-source food consumption.

lowest was found in the two varieties of potatoes and plantain (28–37 mg/100 g).

The highest phytate content was found in cereals: quinoa, wheat grain, maize (1020–2060 mg/100 g) and legumes: peanuts, fava beans and lentils (846–2070 mg/100 g). The phytate level was lower in noodles, wheat flour, rice and bread (99–468 mg/100 g). Regarding tubers, the highest phytate content was found in new cocoyam (427 mg/100 g), followed by potato runa, cassava and potato imilla (77–207 mg/100 g); the phytate in chuño (58 mg/100 g) was the lowest in this group. Phytate content in plantain was the lowest of all (22 mg/100 g).

3.3. Relative mineral bioavailability

The molar ratios, Phy:Zn, Phy:Fe, Phy:Ca and Phy*Ca:Zn are presented in Table 4. Five out of seven cereal foods had molar ratios of Phy:Zn above the critical molar ratio 15 (WHO, 1996), quinoa being the highest among the cereals Phy:Zn (56.5 ± 9.3), followed

by wheat grain (51.5 ± 8.6), and only white bread and rice had Phy:Zn ratios below 15. All legumes had Phy:Zn above 15, with the highest for peanuts (61.5 ± 1.4). Regarding tubers: new cocoyam and potato runa were above 15, but not potato imilla, chuño or cassava; regarding the legumes: fava beans and lentils were above 15. Furthermore, according to the molar ratios Phy*Ca:Zn, the phytate level in white bread is also likely to compromise the zinc absorption with molar ratios >200 (Bindra et al., 1986); only rice, potato imilla, chuño and plantain showed Phy*Ca:Zn below 200. This point to that the zinc bioavailability in most of the analyzed food is compromised by the phytate content.

None of the analyzed food items showed molar ratios of Phy:Fe below 1, the level which is said to be adequate for iron absorption (Hurrell, 2004). The highest Phy:Fe ratios were for peanuts (68.8 ± 2.4), maize (44.4 ± 4.2), wheat grain (36.7 ± 8.1) and quinoa (33.3 ± 8.0), indicating that iron absorption from these foods might be significantly inhibited by phytate content. Regarding the molar ratios of Phy:Ca, ten out of the fifteen food items had Phy:Ca values above the critical molar ratio 0.17 (Umetsu et al., 2005); the highest were found in maize (3.01 ± 0.61), peanuts (2.50 ± 0.16), wheat grain (1.46 ± 0.38) and quinoa (0.72 ± 0.14). Thus, the phytate content in most of the analyzed food was at levels likely to compromise absorption of zinc, iron and calcium markedly.

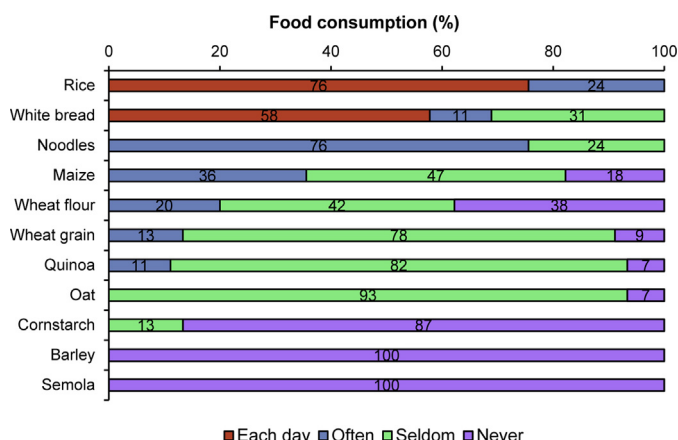


Fig. 2. Frequency of cereals consumption.

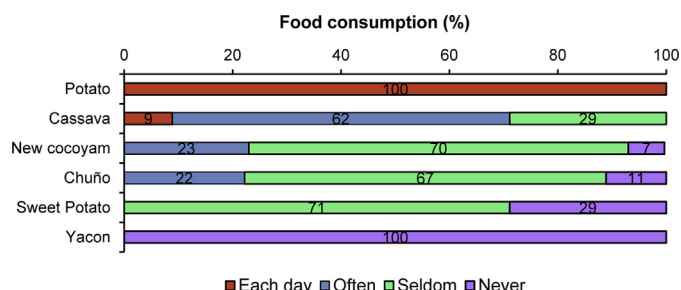


Fig. 3. Frequency of tubers and roots consumption.

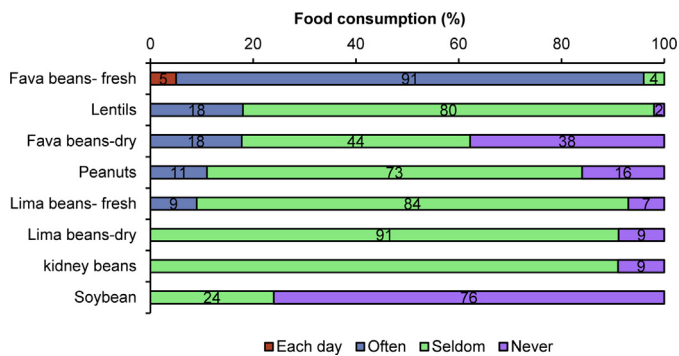


Fig. 4. Frequency of legumes consumption.

4. Discussion

This study provides, for first time, data of phytate and mineral content in the most consumed food in a rural area of Bolivia. These data can also be used at a national level as most of the food analyzed is also part of the habitual diet of the entire Bolivian population (Perez-Cueto et al., 2006). The data is a confirmation of the importance of more analysis of food at national level since there are important discrepancies when comparing to the values presented in USDA reference data and to the Bolivian food composition table, in spite of the missing phytate values in both tables. These data are important for the evaluation of relative mineral bioavailability, useful for highlighting existing mineral

Table 2
Frequency of consumption: vegetables, fruits and fat.

Food item	Frequency %			
	Each day	Often	Seldom	Never
Vegetables				
Onion	100	0	0	0
Carrot	100	0	0	0
Tomato	82	9	9	0
Pepper	62	31	7	0
Cabbage	47	36	18	0
Spinach	9	53	36	2
Onion-green	7	24	58	11
Beetroot	7	20	64	9
Achojcha	5	82	13	0
Green beans	0	87	11	2
Locoto (chili)	0	53	40	7
Avocado	0	31	53	16
Lettuce	0	27	82	0
Chard	0	9	84	7
Cucumber	0	9	84	7
Squash	0	9	84	7
Palm	0	0	7	93
Fruits				
Plantain	7	80	13	0
Orange	0	78	22	0
Banana	0	58	42	0
Tangerine	0	49	51	0
Apple	0	7	89	4
Mango	0	7	71	22
Papaya	0	2	89	9
Pineapple	0	0	100	0
Strawberry	0	0	53	47
Oil and fat				
Vegetable oil	62	34	4	0
Lard	0	20	42	38
Tallow	0	0	62	38
Margarine	0	0	11	89

Most consumed vegetables, fruits and fats in the tropical area Chapare-Bolivia, according to a food frequency questionnaire.

deficiencies and taking decisions concerning the need for fortification or dietary interventions.

It is important to mention that not all of the analyzed food items are produced in the tropical area; in fact, only cassava, new cocoyam and plantain are tropical products. Maize, wheat, fava beans, peanuts and potatoes are mainly produced in the valley; rice and lentils are mainly produced in the lowlands (CIPCA, 2012). Bread is one of the main foods consumed, its preparations may vary slightly from one bakery to another or if it is homemade. However, it follows the same basic process of leavened bread. Chuño is a traditional product derived from potatoes. It is mostly made in the highlands where, during winter, the temperature reaches $\sim -10^{\circ}\text{C}$. The potatoes are spread on the ground and frozen due to the low night temperatures, then during the day, the potatoes are pressed to release some water and left to dry in the sun, the whole procedure lasts for about three weeks and, during this time, the skin of the potatoes is lost (Peñarrieta et al., 2011). Although this process is carried out in the highlands, the popularity of chuño as a habitual food item has spread to the valley and the tropical areas. Another reason for its increasing acceptance is the migration of people from the highlands to the valleys and tropics, and the maintenance of their eating habits. Nowadays, chuño is found in all of the markets around the country.

The FFQ in this study shows that the diets in the rural area of Chapare are based mainly on cereals, tubers and roots, legumes and small contributions of animal-source foods. This is in agreement with previous studies carried out in the same area, where a high-energy consumption from carbohydrates was reported (63–72 E%) (Lazarte et al., 2012, 2013). This type of diet is categorized as a plant-based diet (Gibson et al., 2010). The plant-source foods – cereals, tubers and legumes – not only contribute energy and some protein to the diet, they are also the major sources of essential minerals such as zinc, iron and calcium. However, these foods also contain high levels of phytate. Positive associations were found between the presence of phytate and zinc ($r = 0.714$, $P < 0.01$) or iron ($r = 0.650$, $P < 0.01$), which were stronger than the association between phytate and calcium ($r = 0.415$, $P < 0.01$), which were somewhat more scattered. Cereals and legumes, in particular, have a high content of minerals but also phytates (Sandberg, 2002). In the present study, it was found that phytates are mainly present in cereals, and legumes and, in a lower proportion, in roots and tubers. This is in agreement with other studies (Gibbs et al., 2011; Lönnnerdal, 2000). The results emphasize that quinoa and legumes are the best sources of zinc, iron, and calcium, but caution must be taken with regard to their high level of phytate. Some cereals and legumes also contain high amounts of iron binding polyphenols inhibiting iron absorption (Sandberg, 2002).

In terms of mineral analysis, there are noticeable discrepancies between our results and the values presented in the USDA or Bolivian food composition tables (Table 5). In general, the differences are larger when comparing the content of zinc or calcium than iron. These discrepancies may be due, for example, to differences in soil, cultivars, growing conditions and food preparation practices (Gibson et al., 2010). This confirms the need for representative mineral values, as in this study, for the most consumed food in Bolivia.

Regarding wheat and wheat products, the wheat grain was found to be a good source of iron, zinc and calcium, although the level of phytate is considerably high, 1320 mg/100 g, which is higher than the concentrations (450–640 mg/100 g) found by Eagling et al. (2014). In addition, circumstances like the extraction rate of flour or the baking process of bread influence the phytate concentration. Thus, in the white wheat flour the phytate content was significantly lower than in the wheat grain, in the range from 200 to 316 mg/100 g, and in the lower range of previously reported

Table 3Zinc, iron, calcium and phytate contents of main food consumed in Chapare-Bolivia, mean \pm SD (min. to max.) in dry weight (DW).

Food item	n	Moisture g/100 g	Zinc mg/100 g	Iron mg/100 g	Calcium mg/100 g	Phytate mg/100 g
Wheat grain	10	66.0 \pm 3.4	2.56 \pm 0.24 (2.27–2.98)	3.24 \pm 1.09 (2.19–5.16)	57 \pm 10 (45–74)	1320 \pm 140 (1170–1620)
Wheat flour	10	8.6 \pm 1.0	1.52 \pm 0.22 (1.15–1.78)	5.24 \pm 0.57 (4.59–6.31)	51 \pm 4 (47–58)	272 \pm 41 (200–316)
White bread	10	21.0 \pm 1.4	1.00 \pm 0.09 (0.89–1.16)	5.11 \pm 0.30 (4.45–5.43)	187 \pm 47 (142–248)	99 \pm 20 (78–134)
Maize	10	58.2 \pm 3.5	2.42 \pm 0.39 (2.02–3.16)	1.96 \pm 0.44 (1.57–2.72)	21 \pm 6 (17–28)	1020 \pm 180 (880–1420)
Rice	10	66.5 \pm 3.5	1.64 \pm 0.17 (1.33–1.82)	0.49 \pm 0.45 (0.42–0.55)	27 \pm 15 (15–56)	142 \pm 35 (86–190)
Noodles	10	64.8 \pm 2.0	1.77 \pm 0.43 (1.18–2.28)	4.92 \pm 1.36 (3.20–7.34)	87 \pm 5 (84–97)	468 \pm 47 (369–524)
Quinoa	10	73.1 \pm 2.3	3.65 \pm 0.32 (3.13–4.12)	5.40 \pm 0.84 (3.95–6.27)	176 \pm 19 (144–200)	2060 \pm 230 (1530–2280)
Cassava	10	69.0 \pm 2.2	1.48 \pm 0.25 (1.19–1.93)	0.83 \pm 0.06 (0.74–0.94)	93 \pm 11 (71–110)	199 \pm 70 (115–312)
New cocoyam	10	70.7 \pm 1.2	2.32 \pm 0.82 (1.74–3.96)	1.84 \pm 0.47 (1.31–2.54)	113 \pm 65 (72–236)	427 \pm 95 (275–528)
Potato imilla	10	74.2 \pm 1.6	1.03 \pm 0.13 (0.82–1.19)	1.50 \pm 0.35 (0.83–1.73)	37 \pm 9 (25–48)	77 \pm 29 (59–136)
Potato runa	10	77.0 \pm 1.8	1.13 \pm 0.19 (0.92–1.44)	1.66 \pm 0.23 (1.45–2.17)	37 \pm 13 (19–58)	207 \pm 75 (110–333)
Chuño	10	74.0 \pm 3.4	0.94 \pm 0.16 (0.75–1.30)	2.14 \pm 0.50 (1.22–2.68)	120 \pm 36 (75–173)	58 \pm 8 (47–75)
Fava beans	10	75.0 \pm 1.7	4.64 \pm 0.55 (3.83–5.54)	4.71 \pm 0.47 (3.91–5.56)	115 \pm 21 (83–140)	1170 \pm 320 (840–1690)
Lentils	10	62.6 \pm 3.4	3.49 \pm 0.35 (3.03–4.02)	6.43 \pm 0.18 (6.11–6.67)	131 \pm 18 (109–164)	846 \pm 82 (747–961)
Peanuts	4	3.64 \pm 0.5	3.33 \pm 0.30 (3.04–3.60)	2.54 \pm 0.25 (2.28–2.76)	50 \pm 2 (48–52)	2070 \pm 220 (1860–2310)
Plantain	10	71.7 \pm 2.0	0.75 \pm 0.34 (0.56–1.43)	1.36 \pm 0.29 (1.10–1.84)	41 \pm 8 (28–52)	22 \pm 6 (17–36)

Table 4Molar ratios of phytate*calcium to zinc and phytate to zinc, iron and calcium in most consumed plant-food in Chapare-Bolivia, mean \pm SD (min. to max.), in dry weight (DW).

Food item	Phy*Ca:Zn	Phy:Zn	Phy:Fe	Phy:Ca
Wheat grain	729 \pm 162 (1060–2020)	51.5 \pm 8.6 (42.5–67.9)	36.7 \pm 8.1 (25.6–47.2)	1.46 \pm 0.38 (1.04–2.16)
Wheat flour	226 \pm 21 (401–509)	17.7 \pm 1.3 (15.5–20.2)	4.4 \pm 0.9 (2.9–5.6)	0.32 \pm 0.06 (0.21–0.38)
White bread	458 \pm 166 (323–774)	9.8 \pm 1.9 (6.7–12.5)	1.6 \pm 0.4 (1.2–2.4)	0.03 \pm 0.01 (0.02–0.05)
Maize	222 \pm 62 (303–662)	41.8 \pm 4.5 (35.8–49.9)	44.4 \pm 4.2 (37.4–49.3)	3.01 \pm 0.61 (2.00–3.76)
Rice	53 \pm 21 (38–101)	8.5 \pm 1.6 (5.7–10.7)	24.9 \pm 7.0 (13.3–34.2)	0.41 \pm 0.23 (0.09–0.73)
Noodles	611 \pm 151 (441–856)	27.4 \pm 6.0 (20.7–37.0)	8.7 \pm 2.7 (4.5–12.6)	0.32 \pm 0.03 (0.26–0.36)
Quinoa	2470 \pm 340 (1890–2880)	56.5 \pm 9.3 (40.2–69.6)	33.3 \pm 8.0 (20.6–47.0)	0.72 \pm 0.14 (0.49–0.96)
Cassava	311 \pm 107 (121–491)	13.5 \pm 4.8 (7.8–20.4)	20.5 \pm 8.1 (11.7–35.5)	0.13 \pm 0.05 (0.07–0.21)
New cocoyam	506 \pm 210 (212–895)	20.1 \pm 7.3 (8.3–28.4)	21.6 \pm 8.8 (9.6–31.3)	0.30 \pm 0.14 (0.07–0.41)
Potato imilla	71 \pm 35 (40–134)	7.3 \pm 2.3 (5.2–11.9)	4.59 \pm 1.94 (2.9–7.1)	0.13 \pm 0.03 (0.08–0.18)
Potato runa	179 \pm 119 (68–545)	18.4 \pm 6.5 (9.50–29.0)	10.3 \pm 2.4 (6.2–14.1)	0.37 \pm 0.16 (0.15–0.65)
Chuño	181 \pm 45 (122–258)	6.3 \pm 1.7 (4.1–9.4)	2.4 \pm 0.8 (1.6–3.7)	0.03 \pm 0.01 (0.02–0.06)
Fava beans	717 \pm 208 (972–2070)	25.3 \pm 7.1 (17.4–35.6)	21.0 \pm 4.5 (15.7–26.6)	0.66 \pm 0.27 (0.37–1.04)
Lentils	791 \pm 142 (1240–2120)	24.2 \pm 2.9 (18.5–27.8)	11.1 \pm 0.9 (9.8–12.2)	0.40 \pm 0.07 (0.29–0.50)
Peanuts	772 \pm 48 (726–828)	61.5 \pm 1.4 (60.7–63.6)	68.8 \pm 2.4 (66.2–71.0)	2.50 \pm 0.16 (2.32–2.69)
Plantain	35 \pm 17 (11–61)	3.4 \pm 1.6 (1.2–6.2)	1.4 \pm 0.4 (0.8–1.9)	0.03 \pm 0.01 (0.02–0.05)

values for phytate in wheat flour (154–1750 mg/100 g) (Febles et al., 2002; García-Estapa et al., 1999). The high variability between different wheat flours is above all attributed to the extraction rate of the flours, as phytate is mainly distributed in the external covers, in the pericarp and in the aleurone layer of the wheat; a process like dehulling effectively removes significant amounts of phytates (Majzoubi et al., 2014; Salunke et al., 2012).

However, during dehulling not only phytate is lost but also an important amount of minerals. Thus, white wheat flour, for example, had 40% less zinc than wheat grain. The reason why the iron content was higher in the white wheat flour compared to the whole wheat grain is that, due to the fortification policies in Bolivia, wheat flour must be fortified with iron at a level of 6 mg/100 g (David, 2004). Therefore, Phy:Fe in white wheat flour was as much as 88% lower than in wheat grain. Also, the Phy:Zn and Phy:Ca ratios were lower by 66% and 78% respectively, an indication of a higher mineral bioavailability. Notwithstanding, this reduction in phytate:mineral levels was not enough to reach adequate ratios (Table 3), and wheat flour is also considered a food with low mineral bioavailability.

The concentration of phytate in white wheat bread (99 mg/100 g) was 63% lower than in the white wheat flour (272 mg/100 g); these results are in agreement with previous studies. Ma et al. (2005) reported the phytate concentrations in wheat bread (29.1 mg/100 g) to be 89% lower than in wheat flour (217.87 mg/100 g) and García-Estapa et al. (1999) showed a reduction of 50% (from 298 to 148 mg/100 g) of phytate during the bread making process. The phytate reduction during the breadmaking procedure is attributed to endogenous phytase activity of the wheat flour, which is influenced by acidity of the dough and the temperature and to a minor extent on the action of yeast phytase (Türk and Sandberg, 1992). With sourdough fermentation it is possible to obtain an almost complete degradation of phytate (Larsson and Sandberg, 1991).

Table 5

Comparison of zinc, iron and calcium analyzed in the selected food with values in the nutrient database for standard reference USDA and with the Bolivian food composition table, in dry weight (DW).

Food items	Zinc analyzed	Zinc USDA ^a	Zinc BFCT ^b	Iron analyzed	Iron USDA	Iron BFCT	Calcium analyzed	Calcium USDA	Calcium BFCT
Wheat grain (mg/100 g)	2.56	3.68 (–30)	4.16 (–39)	3.24	5.04 (–36)	3.00 (8)	57	35 (61)	59 (–3)
Wheat flour (mg/100 g)	1.52	0.80 (90)	0.60 (153)	5.24	5.27 (–1)	6.53 (–20)	51	17 (202)	63 (–18)
White bread (mg/100 g)	1.00	1.88 (–47)	2.00 (–50)	5.11	5.38 (–5)	4.00 (28)	187	221 (–16)	46 (302)
Maize (mg/100 g)	2.42	2.47 (–2)	2.00 (21)	1.96	3.02 (–35)	2.80 (–30)	21	8 (174)	13 (60)
Rice (mg/100 g)	1.64	1.34 (22)	1.16 (41)	0.49	4.75 (–90)	1.45 (–66)	27	10 (180)	13 (107)
Noodles (mg/100 g)	1.77	2.01 (–12)	1.00 (77)	4.92	4.56 (8)	3.50 (41)	89	37 (138)	60 (48)
Quinoa (mg/100 g)	3.65	3.83 (–5)	3.10 (18)	5.40	5.25 (3)	5.70 (–5)	176	60 (195)	134 (31)
Cassava (mg/100 g)	1.67	0.84 (99)	0.50 (234)	0.83	1.80 (–54)	1.36 (–39)	93	40 (135)	135 (–30)
New cocoyam (mg/100 g)	2.32	–	–	1.84	–	–	113	–	–
Potato imilla (mg/100 g)	1.03	1.20 (–14)	0.40 (158)	1.50	1.38 (9)	1.23 (22)	37	36 (5)	36 (2.5)
Potato runa (mg/100 g)	1.13	–	–	1.66	–	1.00 (66)	37	–	46 (–19)
Chuño (mg/100 g)	0.94	1.45 (–35)	0.30 (213)	2.14	4.88 (–56)	0.99 (116)	120	41 (194)	51 (134)
Fava beans (mg/100 g)	4.64	3.55 (31)	0.70 (563)	4.71	5.27 (–11)	3.20 (47)	115	127 (–9)	115 (0)
Lentils (mg/100 g)	2.49	4.18 (–40)	1.40 (78)	6.43	10.97 (–41)	3.76 (71)	131	63 (109)	74 (78)
Peanuts (mg/100 g)	3.33	3.36 (–1)	2.93 (14)	2.54	3.23 (–21)	2.29 (11)	50	55 (–8)	54 (–6)
Plantain (mg/100 g)	0.75	0.40 (88)	0.20 (275)	1.36	1.77 (–23)	0.90 (51)	46	6 (648)	18 (161)

Data in mg/100 g and percentage of the difference in parenthesis. Calculated as: (value from the analysis – value from the table) × 100/value from the table.

^a USDA, food composition for cooked food, except cassava that is raw, data extracted from the standard reference from USA (USDA, 2001).

^b BFCT, food composition for raw food, data extracted from the Bolivian food composition table (INLASA, 2005).

Regarding the mineral content in bread, there is a noticeably higher content of calcium in bread (187 mg/100 g) compared to the content in flour (51 mg/100 g), but we do not know for sure the calcium content in the wheat flour used in the different bakeries nor the recipes. As expected, the molar ratios phytate:mineral were lower in bread compared to wheat flour; thus, Phy:Zn was 45% lower, Phy:Fe was 63% lower and Phy:Ca 91% lower in bread, but the ratio of Phy*Ca:Zn was double. The decrease of the molar ratios of zinc and calcium in bread suggests that the absorption of zinc and calcium might not be highly affected by the phytate concentration, while the Phy:Fe, even though it is lower in bread, it is still on a high enough level to compromise the absorption of iron. A virtually complete degradation of phytate is required to substantially improve iron absorption from bread meals (Brune et al., 1992). The molar ratios for noodles, another derived product from wheat flour, are also above the critical levels; Phy:Zn was 27.4, and Phy:Fe and Phy:Ca 8.7 and 0.32 respectively. These values are similar to those previously reported by Ma et al. (2005): 19.8, 7.2 and 0.35 respectively.

The concentration of phytate in maize was high, 1020 mg/100 g; similar results were reported by other authors: 1443 mg/100 g (Abebe et al., 2007) and 744.6 mg/100 g (Chan et al., 2007). Maize had the highest molar ratios: Phy:Zn 41.8, Phy:Fe 44.4 and Phy:Ca 3.01 among the cereals, and high molar ratios were also reported in previous studies, Phy:Zn 35.4, Phy:Fe 27.8 and Phy:Ca 5.45 (Abebe et al., 2007), indicating that the mineral absorption in maize is highly inhibited by the level of phytate.

The concentrations of phytate in rice were the lowest found among the cereals: 142 mg/100 g, which is within the phytate concentrations found by Ma et al. (2005) in 3 varieties of rice (92–183 mg/100 g). The Phy:Zn 8.5 was below the critical value, but Phy:Fe 24.9 and Phy:Ca 0.41 were above the critical values; similar ratios were reported previously for Phy:Zn (range 8.29–11.27), but higher values were found for Phy:Fe (range 40.46–69.67) and Phy:Ca (range 1.18–4.32) (Ma et al., 2005).

Quinoa is a potential source of minerals: zinc 3.65, iron 5.40 and calcium 176 mg/100 g; these values are within the ranges previously presented for six varieties of quinoa in Chile: for zinc (2.73–5.01 mg/100 g), iron (4.82–7.19 mg/100 g) and calcium (77.10–211.9 mg/100 g) (Miranda et al., 2012). The level of phytates in quinoa was the highest among the cereals analyzed in the present study (ranged from 1530 to 2280 mg/100 g). Therefore, the molar ratios were high above the critical values:

Phy:Zn 56.5, Phy:Fe 33.3, Phy:Ca 0.72. There is a scarcity of recent data on the concentration of phytates in quinoa, or on molar ratios, Ruales and Nair (1993) reported phytate concentrations for unpolished quinoa grain (1004 mg/100 g) and polished quinoa grain (780 mg/100 g), it is noticeable that the values obtained in the present study are 2 times higher than those previously reported. Some reasons for these discrepancies are the origin of the plant-food, the different cultivars, variations in the mineral content in the soil and others; besides, the quantification method used in the reference was colorimetric, whereas here we used an HPLC method, well known for its higher sensitivity and accuracy.

Regarding phytates in legumes: peanuts had the highest level (2070 mg/100 g); results are in agreement with earlier reports of 2008 mg/100 g, with high Phy:Zn 60 (Harland et al., 2004), but higher than those reported by Chan et al. (2007) or Mitchikpe et al. (2008) from 483 to 667 mg/100 g. The Phy:Zn in the present study was also very high (61.5), as well as the values of Phy:Fe (68.8) and Phy:Ca (2.50). Phytate contents in lentils (846 mg/100 g) are similar to those reported for four cultivars of lentils (910 mg/100 g) (Wang and Daun, 2006).

Regarding the phytate content in tubers, the highest value was found in the tropical tuber new cocoyam (275–528 mg/100 g), followed by cassava (115–312); these ranges show high variability between the tropical tubers collected from the 5 different villages of Chapare. It is likely that the soil in these areas also presents high mineral variability. Furthermore, data from the literature show wide discrepancies in the phytate concentration in tropical tubers. Similar species to new cocoyam, are yam and taro (cocoyam) lower phytate concentrations were reported for these tubers in the range of 63–105 mg/100 g (Umata et al., 2005); on the other hand, higher values were also reported in the range of 637–855 mg/100 g (Marfo et al., 1990). Phytate content in cassava also varies widely from 95 to 624 mg/100 g (Charles et al., 2005; Marfo et al., 1990); the method of analysis used in these references was colorimetric. However, Mitchikpe et al. (2008) reported no detectable values for cassava and yam (in dried chips) in Northern Benin by the HPLC method. Thus, for tubers and roots where the composition will be highly affected by the soil, is not possible to use general values of phytate content, as the variation is high.

Phytate content was the lowest in potatoes: imilla (77 mg/100 g) and runa (207 mg/100 g). Moreover, here we present the first data of phytate content in the traditional freeze sun-dried potato named chuño, which resulted in a content between 25 and

72% lower than that found in potatoes. However, it is unknown whether the same varieties of potato were used to prepare the chuño samples that were analyzed. This calls attention to the traditional process of obtaining chuño that can be considered as a dietary strategy to reduce the phytate content in potatoes and other tubers. Therefore, it is interesting to consider further research to evaluate the actual phytate reduction during the chuño process. Regarding the mineral content in chuño, it is also expected that some minerals would be lost during the process by leaching with the water, which might be the same via for phytate losses. The results showed that zinc concentration in chuño (0.94 mg/100 g) was lower than in potatoes (1.03–1.13 mg/100 g), but iron is approximately 2 times higher (increases from 1.50 to 2.14 mg/100 g) and calcium 3 times higher (increases from 37 to 120 mg/100 g) than the concentration in potatoes. These results are in agreement with those found by Burgos et al. (2009), reporting that during chuño processing there was a decrease in zinc concentration (from 2.15 to 0.45 mg/100 g), and an increase in iron concentration (from 2.37 to 2.80 mg/100 g) as well as in calcium (from 32.27 to 121.05 mg/100 g) in dry weight. The increase in mineral content may be due to soil contamination during the process.

5. Conclusion

It is of great importance to take into account the phytate content of foods when conducting dietary evaluations and further dietary interventions. As indicated in the present paper, within the Bolivian diet there are foods considered good sources of minerals, such as quinoa, wheat, maize and legumes. However, caution must be taken with the high concentrations of phytate, which might significantly decrease the bioavailability of zinc, iron and calcium as it is shown by the molar ratios. Phytate may be one of the main factors leading to deficiencies of zinc and iron, especially in populations where these plant foods are an important part of the diet. Therefore, nutritional strategies including germination, soaking or fermentation are advised as they have been proved to reduce the content of phytate and enhance the mineral bioavailability (Sandberg and Andlid, 2002). These procedures are important, not only in Chapare, but also in other rural areas in developing countries, where animal sources of food are limited.

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